ULTRAVIOLET LIGHT-INDUCED PURINE MODIFIED DNA

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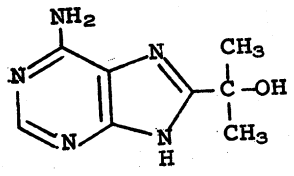
SUMMARY

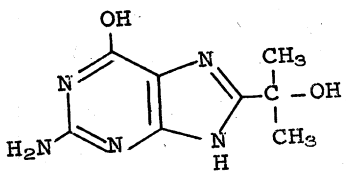
Sensitized or direct ultraviolet light irradiation of DNA in the presence of 2-propanol induces photochemical changes in the purine moieties. The resulting purine photoproducts have chromatographic mobilities similar to the C-8 hydroxyalkyl derivatives of adenine and guanine.

The photochemical reactions of the pyrimidine bases in DNA have been studied in detail and a correlation between pyrimidine dimers and biological activity has been established (1,2 and references cited therein). On the other hand, little information concerning the effect of irradiation on the purine moieties in nucleic acids is presently available. The purines, like the pyrimidine bases, serve as part of the light absorbing system in nucleic acids; however, instead of undergoing photochemical alterations the purine moieties in DNA have been reported to participate mainly in energy transfer processes (3,4).

Recent studies have shown that purines and the corresponding nucleosides undergo photochemical reactions with alcohols (5-7). The products formed in the reactions between adenine or guanine and 2-propanol have been identified as the corresponding C-8 hydroxyalkylpurines.



C-8 hydroxyalkyladenine



C-8 hydroxyalkylguanine

We report here that ultraviolet light irradiation in the presence of 2-propanol modifies the purine bases in DNA.

EXPERIMENTAL

Escherichia coli M55-B46, a purine requiring mutant, was grown in M-9 minimal medium supplemented with 1 μg/ml 8-[^{14}C]-adenine (0.3 μc/ml). DNA was isolated by a modification of the method of Thomas *et al*. (8) and had a final specific activity of 1.2×10^4 cpm/μg.

Irradiation was carried out at room temperature using a Hanovia 200 W high pressure mercury vapor lamp. For direct light irradiation the DNA samples (1 ml) were irradiated in quartz tubes of 1 cm diameter through Corex filters ($\lambda > 260$ nm), while Pyrex filters, which transmit wavelengths longer than 290 nm, were used for the acetone-sensitized reaction. Prior to irradiation the samples were flushed for 10 min with oxygen-free nitrogen.

After irradiation all DNA samples were dialysed for 24 hours against 0.1 M phosphate buffer (pH 8) and the purine bases in DNA were liberated by mild acid treatment (1 ml 2% HCl at 100°C for 10 min) and chromatographed (ascending) in n-butanol-water-ammonia (86:13:1 v/v). The radiochromatograms were analysed as previously described (9). The location of co-chromatographed authentic samples of the C-8 hydroxyalkyl derivatives of adenine and guanine, prepared as described by Steinmaus *et al*. (7), was detected with a Mineralight lamp.

RESULTS AND DISCUSSION

[^{14}C]-Purine labelled *E. coli* DNA was irradiated with UV light in the presence of 2-propanol. The chromatographic distribution of the purines in irradiated DNA is shown in Fig. 1a. It is seen that in addition to guanine and adenine two [^{14}C]-purine derived photoproducts have chromatographic mobilities similar to those of authentic samples of the appropriate C-8 hydroxyalkylguanine (G_1) and C-8 hydroxyalkyladenine (A_1). Under the con-

ditions of irradiation used in Fig. 1a, adenine as well as guanine were converted to the corresponding photoproducts to the extent of 2.6 percent.

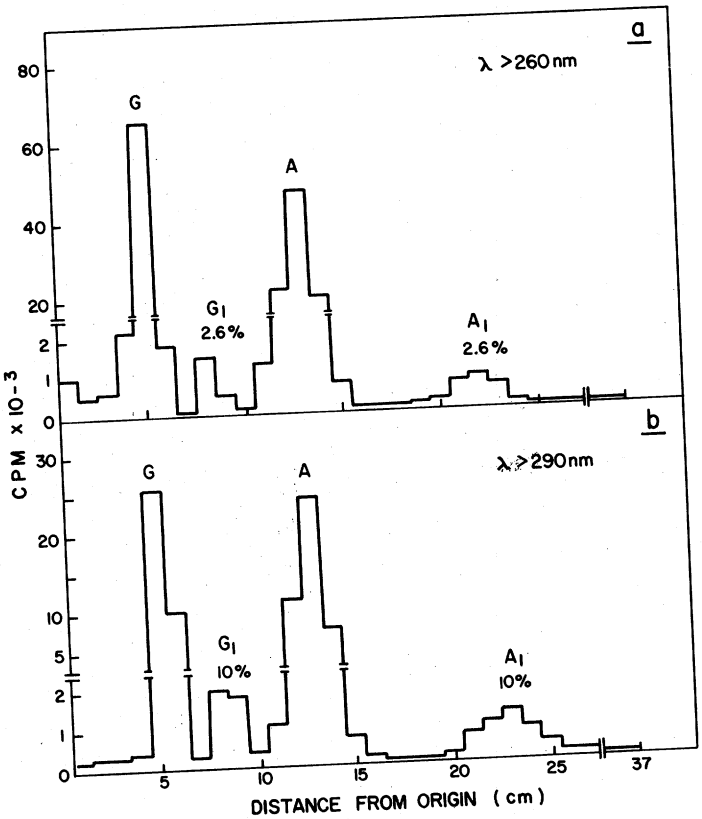


Fig.1. Chromatographic mobilities of purine photoproducts in DNA.
a) [^{14}C]-Purine labelled *E. coli* **DNA (12.5 μg/ml) was irradiated with direct UV light for 30 min (incident light 5.10^{-6} einstein. $cm^{-2}.min^{-1}$) in the presence of 0.5M 2-propanol.**
b) Labelled DNA (8 μg/ml) was irradiated at λ > 290 nm for 30 min (incident light $4.4.10^{-6}$ einstein $\cdot cm^{-2}.min^{-1}$) in the presence of 0.5M acetone and 0.5M 2-propanol.
G,A,G_1 and A_1 denote position of guanine, adenine and co-chromatographed authentic samples of C-8 hydroxyalkylguanine (Rf 0.22) and C-8 hydroxyalkyladenine (Rf 0.60), respectively. Percentage of conversion of guanine and adenine to the corresponding photoproducts is identical.

The same photochemical reaction of purines in DNA was also observed when the nucleic acid was irradiated at wavelengths greater than 290 nm in the presence of 2-propanol and acetone,

the latter serving as the photosensitizer (Fig. 1b). No purine photoproducts were detected when DNA was irradiated with direct UV light in the absence of 2-propanol, or when irradiation at wavelengths greater than 290 nm was carried out in the absence of either acetone or 2-propanol.

In the experiments described irradiated DNA was extensively dialysed after irradiation. During dialysis, or after alcohol precipitation of DNA, no loss of [^{14}C] radioactivity or change in the chromatographic distribution of the purine photoproducts was detected, indicating that the photoproducts formed in DNA are not split off during the photochemical reaction or subsequent dialysis.

It has previously been shown that thymine in DNA can be dimerized by irradiation at wavelengths greater than 290 nm using acetone as photosensitizer (10). Using [^{14}C] labelled DNA, we recently observed that sensitized irradiation in the presence of 2-propanol decreases thymine dimerization to one third of that obtained in the absence of the alcohol.

The results presented demonstrate that purines in DNA react photochemically with 2-propanol. The photoproducts produced have chromatographic mobilities similar to those of the appropriate C-8 hydroxyalkyl derivatives of guanine and adenine. Definite proof of the identity of the purine photoproducts with the hydroxyalkylated bases have, however, not yet been obtained. It is assumed that 2-propanol acts as a hydrogen donor to the excited DNA (11,12) in the direct light induced reaction, or to the excited sensitizer in the photosensitized one. As a result of these interactions ketyl radicals [$(CH_3)_2\dot{C}OH$] are formed and these are subsequently scavenged by the purines in DNA to produce C-8 hydroxyalkyl derivatives (13).

Since 2-propanol serves as a model for a variety of other potential hydrogen donors, such as sugars and amino acids, it is possible that the photochemical reaction described may take place in biological systems.

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